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E2F and Cell Proliferation: A World Turned Upside Down

Minireview

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The cottage industry of inactivating genes in the mouse germline is growing by leaps and bounds. However, the making of such mice hardly offers a guaranteed route to interesting results. Often, the phenotypes of the genetically altered mice reinforce already established concepts or are uninformative because of early embryonic lethality. On occasion, though, knockout mice do indeed tell us something novel and fully unexpected. Two reports in this issue describe mutant mice with phenotypes that are precisely opposite to those predicted by conventional wisdom (Field et al., 1996; Yamasaki et al., 1996).

Powerful Transcription Factors

The E2F transcription factors - the objects of study in these knockout mice - play an especially influential role in advancing mammalian cells through their growth cycles. The term "E2F" actually subsumes a group of five closely related proteins (E2F-1 through −5) and a set of partner proteins, members of the DP family of transcription factors. Heterodimers of E2F and DP proteins assemble in various combinations to form functionally active DNA-binding complexes. These are important for regulating gene expression in the G1 phase of the cell cycle and perhaps in later phases as well.

The E2F factors operate under the direct control of the cell-cycle clock machinery. In mid/late G1, D-type cyclins and cyclin E, acting together with cyclin-dependent kinases, phosphorylate the retinoblastoma protein (pRB) and its two cousins, p107 and p130. These three proteins, which have affinity for binding various E2Fs, respond to the phosphorylation by releasing the bound transcription factors; the liberated E2Fs then proceed to trigger expression of genes that enable the cell's advance into late G1 and S phases (Sherr, 1994; Weinberg, 1995). Viewed in this way, pRB and its cousins represent an interface between the core cell cycle clock machinery and the cell's transcriptional apparatus.

While the above description is correct in outline, it glosses over important details. E2Fs-1 through −3 prefer to associate with pRB, while the last two (E2F-4 and −5) prefer liaisons with p107 and p130. Also, the association of pRB and its cousins with the E2Fs can occur while the latter are bound to DNA sites found in a variety of promoters. The resulting complexes often actively repress utilization of these promoters rather than simply occluding the transcription-inducing domains of the E2Fs. As a consequence, the influence of an E2F site on the expression of a gene may actually be a repressive one (Weintraub et al., 1992; LaThangue, 1994).

Accumulating evidence suggests that pRB and possibly its cousins reversibly associate with a number of other proteins besides the E2Fs. But the E2Fs seem to be especially important. Their preeminent role is indicated by a simple experiment: cells that are held in quiescence by serum starvation can be driven all the way through G1 into S by ectopic expression of an E2F, specifically E2F-1 (Johnson et al., 1993; Qin et al., 1994). This unusual trait - the ability of a single transcription factor to drive progression all the way through G1 - is shared, to this writer's knowledge, with only a single other transcription factor, the MYC oncoprotein. Hence, the E2Fs and MYC would appear to sit high in the hierarchy that orchestrates the complex succession of events that represents the G1 phase.

Topsy-Turvy Mice

The two groups that undertook to inactivate the E2F-1 gene in the mouse germline had every right to expect some interesting phenotypes. The fact that there are five E2Fs suggests a measure of functional redundancy, reducing the specter of uninformative, early embryonic lethality. Perhaps, they thought, that the various E2Fs (or at least the three closely allied E2Fs-1,-2 and −3) would be used in different tissues to differing extents, yielding tissue-specific developmental effects when one or another E2F gene was inactivated in the germline.

The expected outcomes were clear: since E2Fs drive cell cycle advance, their absence should yield underdeveloped or even absent tissues. In fact, few if any of these outcomes were observed. The testes in the E2F$^{-/-}$ mice were initially normal but then atrophied with advancing age (Yamasaki et al., 1996; Field et al., 1996). This atrophy would seem to be due to still uncharacterized defects in the elaboration of or response to specific hormones such as testosterone. Such aberrations may or may not be connected with the ability of certain cell types to proliferate properly.

Certain exocrine glands were dysplastic, in that cells of these tissues seem to have passed through aberrant cell cycles, as evidenced by their large size and occasional binucleate appearance (Yamasaki et al., 1996) This too is hardly indicative of a hypoproliferative state. So, clear signs of the expected proliferative failure were not seen.

Instead, a fully unanticipated outcome was observed as these mice aged: some of their tissues began to exhibit hyperplasia and even neoplasia. One research group reports the thymuses of 4-6 week old E2F-1$^{-/-}$ mice are enlarged due to an excess of immature T cells (Field et al., 1996); the other describes a more systemic lymphoproliferative disorder (Yamasaki et al., 1996). In older mice, a substantial increase in the mitotic rate of cells in their thymic cortex becomes apparent. Yamasaki and collaborators describe an equally unexpected and dramatic finding in aging mutant mice: many exhibit a range of tumors, including notably unusual sarcomas of the reproductive tract, lung tumors, and lymphomas. Hence, most of the observed consequences of E2F-1 loss are directly opposite to those that were expected.

Counter-intuitive results like these are the stuff of great science, because they force us to re-examine and revise our paradigms. Unfortunately, mice do not always give us clear guidance into how we should rethink our mechanistic models. The powers of the mouse genetics used here are counter-balanced by its pitfalls. Altering

the genes of a mouse allows one to rise above the narrow arena of gene-cell interaction to view the larger and more interesting interplay between genes and tissues. At the same time, the complexity of tissue physiology often deprives one of clear insight, if only because a number of alternative mechanistic models become plausible.

Two Rationales

The unexpectedly high proliferation of several cell types in these knockout mice can be rationalized by two classes of conceptual models. The first class proposes that the observed effects are cell-autonomous. Thus, the cells that have become hyperplastic and then cancerous have done so because a critical component (E2F-1) of their growth-regulating circuitry has been deleted, resulting in their inability to make appropriate decisions about their own growth, apoptosis, or post-mitotic differentiation.

The other, non-cell-autonomous model, equally plausible a priori, states that the cell populations in these mice that were hyperplastic (and later neoplastic) began to grow abnormally because of defects in their environment. For example, the heterotypic interactions between dissimilar cell types in a tissue often include the exchange of growth-inhibitory signals. If one cell type is responsible for suppressing the proliferation of a neighboring cell layer, then the absence of the first may permit the second to initiate uncontrolled growth. Hence, the observed runaway proliferation in certain tissues of the E2F-1$^{-/-}$ mice may be due to the underdevelopment of tissues normally responsible for releasing growth-inhibitory signals. This line of thinking clings, perhaps unrealistically, to the notion that many of the effects of E2F-1 deletion will ultimately be explainable in terms of the inability of certain cell types to proliferate.

The authors of both reports limit their mechanistic speculations to the first class of models involving cell-autonomous effects. The available evidence supporting one or the other side of this argument comes from the single observation that the cultured cortical thymocytes from the E2F-1$^{-/-}$ mice are less susceptible to apoptosis in vitro than are their wild-type counterparts. This favors cell-autonomous thinking but still does not prove it.

Genes and Tissues

Like many who are altering the mouse germline, the authors of these two reports find themselves gazing across a wide and deep chasm. Standing on one side, they have relatively secure footing in the molecular biology and biochemistry of their genes and proteins and the effects that their genes exert on cell physiology. At great distance on the other side are the complex effects on tissue and organismic phenotype created by their gene alterations. Building a bridge across this chasm will be very challenging. It will likely be a number of years before we understand with any precision why E2F-1 inactivation leads to many of the phenotypes described here.

The authors note an interesting symmetry. The E2F transcription factors can participate in two diametrically opposite effects on transcription. As described above, when complexed directly to DNA in the absence of pRB, the E2Fs can act as strong inducers of transcription; when pRB associates with a DNA-bound E2F, it can actively repress transcription. At the same time, they note the two countervailing effects on cell proliferation: ectopically expressed E2F-1 can drive cells through G1 and, once they have moved into S phase, cause cells to become apoptotic (Johnson et al., 1993; Qin et al., 1994). Hence, E2F can provoke cell proliferation and can also cause the demise of a cell, thereby neutralizing any mitogenic effects that it or other signals have exerted.

Given these opposing effects, a variety of speculations become possible, but they remain nothing more than that. For example, the observation that E2F-1, when ectopically expressed, causes cultured cells to undergo apoptosis, may or may not mean that cells normally modulate their own endogenous E2F-1 expression as a means of inducing their own apoptosis. Such a mechanism could be invoked to explain the reduced tendency to apoptose seen in the E2F-1$^{-/-}$ lymphocytes reported in one of these two papers (Field et al., 1996).

The fact that E2F can serve to repress transcription by attracting pRB might imply that a prime effect of such repression is to shut down cellular growth-promoting genes. This in turn could explain why the deletion of E2F-1 leads to hyperplasia. But this mechanistic model seems a bit too glib. After all, it is just as plausible that E2F-1 plays an even more important role in repressing growth-inhibitory genes, leading to precisely opposite effects when deleted from the cell's repertoire of transcriptional regulators.

Name Calling

Finally, there is the issue of nomenclature. E2F-1 is clearly an oncogene; when co-expressed with other known oncogenes, it leads to cellular transformation, thereby conforming to a widely accepted operational definition of an oncogene (Johnson et al., 1994; Singh et al., 1994, Xu et al., 1995). But the papers in this issue of *Cell* show that E2F-1 gene deletion also leads to cancer. In this sense, E2F-1 is also a tumor suppressor gene, indeed the first gene to claim membership in the two gene classes. Is this a nomenclatural sleight of hand, or does E2F-1 (and perhaps its sibs in the E2F family) really have a foot in both warring camps?

The term "tumor suppressor gene" is often abused. The literature contains a number of reports in which the growth-inhibitory effects of certain genes are described following the ectopic expression of these genes in one or another type of cancer cell. A frequent conclusion is that the genes under study are functioning as tumor suppressors. In truth, an observation of a gene's growth-inhibitory powers says rather little about its normal physiologic role. After all, almost any gene will create some functional imbalance in a cell and slow down growth when it is ectopically expressed at high enough levels. Some oncogenes will even shut down cell growth.

The present papers embrace a more useful and credible operational definition of a tumor suppressor gene: a gene which, when deleted from the genome of a cell or organism, encourages the appearance of a tumor. The two papers in this issue of *Cell* would seem to have satisfied these criteria by studying the effects of germline E2F-1 gene alteration on tumor susceptibility and by analyzing the growth properties of E2F$^{-/-}$ thymocytes in culture.

Many of the tumor suppressor genes studied to date can cause cancer predisposition when present in the germline as mutant alleles in a heterozygous configuration. To be sure, the great bulk of the hyperplastic and neoplastic outcomes reported in these papers are associated with germline homozygosity of null alleles at the E2F-1 locus. But significantly, several of the mice that were heterozygous for an inactive allele of E2F-1 developed tumors similar to those seen in the homozygotes. By this criterion, germline null alleles of E2F-1 parallel the behavior of other known tumor suppressor genes.

The slightly reduced ability of E2F-1$^{-/-}$ thymocytes to enter apoptosis in vitro would also seem to support the candidacy of E2F-1 as a tumor suppressor that operates on a cell autonomous basis. Here, however, there are alternative explanations: perhaps these E2F$^{-/-}$ thymocytes have not been allowed by their in vivo environment to differentiate in precisely the same way as their normal counterparts and therefore may not have developed to a state where they have acquired equal susceptibility to apoptosis. Still, these papers direct our minds to a new way of conceptualizing cellular growth control. Controllers may not be simply promoters or inhibitors of proliferation. Instead, as the authors of these reports would suggest, a single protein may act as either depending on its concentration in the cell.

Selected Reading

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